OBSERVATIONS ON CERCARIÆUM LINTONI MILLER AND NORTHUP AND ITS METACERCARIAL DEVELOPMENT

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Introduction

This species was first recorded by Linton (1915), who found it in *Nassa* (=*Illynassa*) *obsoleta* (Say). He gave a brief description of the sporocyst and of the cercariæum, although he did not name the species. His account was confirmed and extended by Miller and Northup (1926), who named the larva in honor of Professor Linton. These authors made a twelve-month survey of the infestation of *N. obsoleta* with larval trematodes and found *Cercariæum lintoni* emerging at all periods of the year, with the greatest infestation in June and December. In a recent paper Africa (1930) described the excretory system of the cercariæum. He gave a figure of the flame cell pattern and a discussion of the mechanism by which the bladder and its accessory sphincter appear to function.

The present report contains the results of work done at the Marine Biological Laboratory during the summer months of 1930 and 1931. The study of marine cercariæ was suggested by Professor H. W. Stunkard, to whom I am indebted for suggestions and advice during the course of the work and in the preparation of this paper. Grateful acknowledgments are made also of assistance from Professor Edwin Linton, Professor P. W. Whiting, and Mr. Raymond M. Cable.

Material and Methods

Great numbers of snails, *Nassa obsoleta*, were obtained from Quisset Harbor and examined by the isolation method for specimens from which *Cercariæum lintoni* were emerging. These infected snails were placed in glass dishes where it was possible to keep them for days and even weeks. A daily change of water sufficed to maintain the snails in good condition and afforded a constant supply of these larvæ for experimental purposes. Morphological details were studied according to the methods discussed by Stunkard (1930). Compressed, unstained, living cercariæ were observed for details of the flame cell pattern. Others, after staining with neutral red, were used in the study of the different glands. Permanent mounts were made also of specimens fixed and stained by various methods.

Experiments were carried on to determine the effects of changes in environmental conditions, and others were performed in an attempt to elucidate the life history and secure the adult form of the species. Certain features of the cercariæ, (1) the presence of a stylet, (2) presence of cephalic, probably penetration glands, (3) failure to encyst on the walls of the container, and (4) great endurance of changes in environmental factors, appeared to be of significance for developmental studies and suggested that they encyst in a second intermediate host. Accordingly, various invertebrates were exposed to active cercariæ to find out whether penetration and encystment would occur.

Table I

Measurements of Cercariæum lintoni. All measurements are given in millimeters, and each is the average of several measurements. The figures of Linton and Shaw are for living specimens, while those of Miller and Northup are for fixed specimens.

	Linton	Miller and Northup	Shaw
Length	.327	.23	.355
Width, anterior	.06		
" middle	.123	.084	.118
" posterior	.06		
Oral sucker (diameter)	.043		.05
Ventral sucker (diameter)	.0623		.07
Excretory vesicle, length			.07
" " width			.045
Pharynx, length			.025
" width			.022
Stylet, length	.02		.0186

Morphology of the Larvæ

Sporocyst Stage

The appearance, form, and size of the sporocysts were reported by Linton. He found them to average 0.645 mm. in length, 0.272 mm. in width, and to contain 2 to 40 cercariæ per sporocyst. My observations are in substantial agreement. There are about 18 to 20 cercariæ in a medium-sized sporocyst and their arrangement is shown in Fig. 3. Encystment of the cercariæ within the sporocyst was never observed.

Cercariæum

As noted by Linton, the cercariæ manifest much difference of form in extension and contraction. They are elongate forms, usually ovate, with the greatest width at the region of the ventral sucker or just posterior to it. Usually the anterior end narrows gradually and the posterior tip is slightly angular. A specimen full extended measured 0.83 mm. in length and 0.055 mm. in greatest width, while in a contracted condition it was only 0.25 mm. long. Table I gives measurements of the larva and certain of its structures as recorded by different authors.

The entire surface of the body is covered with minute spines; those of the anterior region are somewhat larger than those farther posteriad. They are set in rows, approximately 250 in number, arranged in alternate fashion as indicated in Fig. 9. Linton reported that the anterior spine (stylet) is not easily seen in mounted specimens, while Miller and

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PLATE I

Explanation of Abbreviations in Figures

c—cercariæum
cp—cirrus pouch
dg—ducts of cephalic glands
e—esophagus
ec—encysted cercariæum
ed—ejaculatory duct
es—excretory sphincter
et—lateral excretory tubule
ev—excretory vesicle
g—cephalic gland
gl—gland posterior to ventral sucker
gp—genital pore
ic—immature cercariæum
icw—inner cystic wall
inc—intestinal cecum
m—metraterm
o—ovary
ocw—outer cystic wall
og—opening of cephalic gland duct
ogl—opening of duct of gland posterior to ventral sucker
os—oral sucker
ov—oviduct
p—pharynx
pp—prepharynx
s—stylet
sr—seminal receptacle
sv—seminal vesicle
t—testis
u—uterus
vd—vas deferens
vs—ventral sucker

Explanation of Figures

Fig. 1. *Cercariæum lintoni* Miller and Northup, ventral view.

Fig. 2. Anterior portion of *C. lintoni* showing the relationship of the stylet and the ducts of the cephalic glands when the stylet is drawn within the oral sucker.

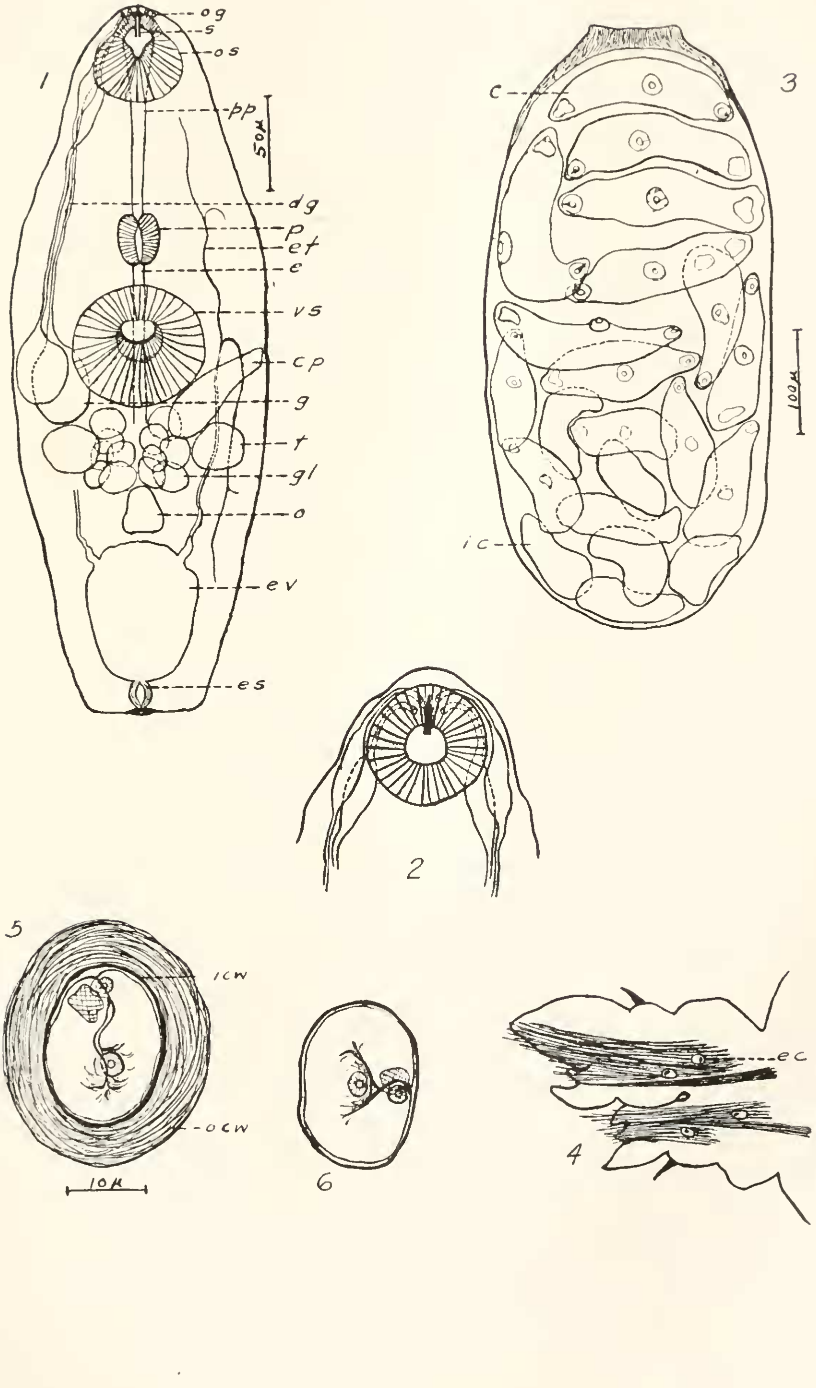
Fig. 3. Diagram of a sporocyst of *C. lintoni*. Note the relative positions of mature and immature cercariæ.

Fig. 4. Diagrammatic drawing of a parapodium of *Nereis virens* Sars., showing the characteristic location of cysts.

Fig. 5. Camera lucida drawing of a ten-day-old cyst of *C. lintoni*. Note the two layers of the cyst wall.

Fig. 6. Camera lucida drawing of the same ten-day-old (Fig. 5) cyst with the cercariæum in a different position.

Plate I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Northup merely sketched it in their figure without mentioning it in their description. It is prominent in all active, living specimens and is shown, much as Africa (1930) figured it, in Figs. 1 and 2.

All accounts of species agree as to the nature and location of the prepharynx and pharynx, but there is considerable disagreement as to the intestine. Linton stated that, " intestinal rami were not distinctly shown " while Miller and Northup reported " very narrow intestinal ceca, traceable only in serial sections, reaching almost to the excretory vesicle." Miller and Northup, and Africa, figure a bifurcation of the short esophagus at the anterior margin of the ventral sucker and slender intestinal ceca which extend caudally. The writer was unable to distinguish such intestinal ceca, and study of later stages indicates that they probably do not exist.

The description of the excretory system, begun by Linton and emended by Miller and Northup, was completed by Africa. He gave the excretory formula as $2[(2+2)+(2+2)] = 16$ flame cells, and figured the flame cells with connecting tubules. My observations are in full accord and verify his conception of certain connecting tubules of which he was not positive.

Rudiments of the reproductive organs were observed by Linton and also by Miller and Northup. Linton's observation concerning the testes is confirmed but the structures he identified as the rudimentary ovary and the " beginning of the uterus " have been shown in the present study to be the beginnings of the cirrus sac and the ovary respectively. He suggested that certain granular masses might be the " beginnings of diffuse vitellaria," but this point is still uncertain.

The two pairs of cephalic glands shown in Fig. 1 have been described by previous workers. But in addition, by intra-vitam staining with neutral red, I have been able to recognize six pairs of glands in the region just posterior to the ventral sucker.

Experimental Studies

Environmental Effects

The effects of various dilutions of sea water on the activity and longevity of these cercariæ were reported (Stunkard and Shaw, 1931). Specimens placed in 50 per cent and 25 per cent sea water remained alive as long or even a few hours longer than those in undiluted sea water. Room temperature was used in all of these experiments. The cercariæ lived, under these conditions, for 36 to 70 hours.

The cercariæ were much more resistant than those of other species, such as *Cercaria quissetensis* Miller and Northup (from the same host), on exposure to low temperatures. A dish containing hundreds of recently emerged specimens was placed at a temperature of 4° to 6° C. The early reaction was an increase in activity, but an hour later they were less active and there was a gradual decrease of activity during the remainder of the lifetime. By the eighth day a few had died, by the tenth day the majority were dead, and only a few showed slight activity on the twelfth day. The same experiment with *Cercaria quissetensis* showed that species to be much less resistant; each cercaria had ceased all activity by the end of the fourth day.

Infection Experiments

Since the cercariæ are tailless, they are unable to swim and an intermediate host must be present in the immediate vicinity of the snail from which they emerge. Miller and Northup described their activity as, "inch-worm locomotion . . . by successive attachment of posterior end of body, modified by invagination into an adhesive disc, then extension of body and attachment by ventral sucker." Very rarely one was found that had encysted on the side of the dish, but usually they showed gradual decrease in activity unto the point of death.

Nereis virens Sars. was successfully infected in the first attempt and gave positive results in all later experiments. The worm was placed in a bowl of sea water containing several hundred recently emerged cercariæ. After an exposure of from two to five hours it was removed to a specimen bowl, which contained well washed sand one-half inch deep. The bowl was then covered with a piece of scrim cloth held in place by a rubber band and placed under a slowly running stream of sea water. In this manner the infected worm could be kept for several weeks. It was interesting to note that cysts were never found in any part of the host other than the parapodia. Their typical location in the parapodium is indicated in Fig. 4; no doubt the rich blood supply in this region affords especially favorable conditions for development of the metacercariæ. At frequent intervals, parapodia were clipped off and the cysts dissected out and studied. The metacercariæ were removed by slightly crushing, or dissecting the cysts, and studied by the same methods used for the cercariæ.

Various invertebrates, including other annelids (*Hydroides* sp., *Lumbrinereis hebes, Scoloplos robustus,* and *Arabella opalina*) [1] and the flatworm, *Bdelloura candida,* were used in similar studies. These species were selected since they may occur in the same habitat as *Nassa obsoleta* and conceivably might serve as hosts of the parasite. In each instance the cercariæ crawled over the surface of the exposed animal and a few penetrated the body wall and encysted. Such cercariæ, when

[1] The author is endebted to Dr. L. P. Sayles, Department of Biology, College of City of New York, for identification of the last three forms.

removed from their cysts, did not manifest the activity and developmental changes that characterize metacercariæ dissected from cysts in *Nereis virens*. In no other host did development occur; on the contrary the larvæ died and the cysts became brown and opaque, according to numerous examinations.

To observe the method of penetration, parapodia were cut from non-infected *Nereis* and placed in Syracuse watch glasses with many active cercariæ. In a few moments the cercariæ were crawling over the parapodia. An hour later several were in very much contracted form and seemed to be inactive but attached to the parapodia. A short time later some of these worms, with anterior end against the parapodium, showed actual "pecking" and boring movements. At the end of two hours some had the anterior end well buried in the parapodium with the caudal end showing great muscular activity. At this time one was observed to be buried in the tissue with only the extreme posterior tip free. One hour later, examination with the binocular showed some within the parapodia and with walls around them. Since the parapodia began to show signs of disintegration at this point the study was not carried farther. It seems quite evident that the cercariæ actually bore their way into the fleshy parapodia by means of the stylet, and presumably the secretion of certain glands, notably the cephalic glands, aids in the process.

Nereis virens has proved to be an extraordinarily convenient host for such a study. As stated above, the experimental method of infestation was very simple since all that was necessary was to isolate a worm in a dish with hundreds of active cercariæ for two to five hours. Each time such an exposure was made the majority of parapodia, examined later, contained from one to six cysts—the results of the penetration and encystment of as many cercariæ. The infected *Nereis* could be kept alive for weeks and did not seem to suffer from the periodic clipping of its parapodia.

Development of the Metacercaria in Nereis

The development of the metacercaria within its cysts is typical, since there is a gradual reduction of larval structures with concomitant growth and differentiation of the organs of the adult stage, which is yet unknown. The transformation of the larva from cercaria to metacercaria is shown in Figs. 6, 7, 8, 10, and 11.

The Cyst

The cysts may be readily observed in the parapodia with a binocular microscope. The wall of the cyst is quite transparent in early stages and never becomes truly opaque, even in later stages, as long as the metacercaria is alive. In older stages the cyst wall consists of two coats, as shown in Fig. 5. The inner coat, which is formed from glandular material of the larva, is thin, clear, and easily ruptured. Around it there develops a thick coat of connective tissue, produced as a reaction of the surrounding tissues of the parapodium, which is tough and difficult to break. Pressure on the cyst causes the contained worm to squirm and it is possible to observe certain features very clearly. The excretory vesicle is especially prominent, as it is filled with concretions that make it appear dark. The cysts increase in size as the metacercariæ develop; Table II summarizes measurements made of cysts of various ages.

Loss of Larval Structures

The large cephalic, or penetration glands are resorbed and have disappeared entirely by the sixth day. The stylet is clearly visible and

TABLE II

Measurements of cysts of Cercariæum lintoni M. and N. which had been dissected out of Nereis. Each figure is the average of eight or more measurements made of forms in an uncovered drop of water.

Age	Length	Width
days	*mm.*	*mm.*
4	.15	.13
13	.21	.155
26	.246	.202
36	.258	.191
45	.26	.225

apparently unchanged during the first ten days of the encysted stage. By the thirteenth day it is reduced in size and the form is somewhat modified as the tip is very blunt. In the cercarial stage the stylet measures 0.0186 mm. in length and 0.033 mm. in width. In the thirteen-day-old metacercaria it measures 0.0156 by 0.0022 mm.; in the twenty-two-day-old 0.0066 by 0.002 mm.; and in the twenty-six-day-old the stylet is lacking completely.

Development of Adult Characters

Certain structures, e.g. the details of the excretory system, are established in the cercarial stage and seem to remain constant throughout development. But the digestive system and the reproductive organs become more developed, or at least more evident, as the metacercariæ increase in age and size.

As discussed previously, the exact nature of the digestive system in the cercarial stage is doubtful. The prepharynx is long with the pharynx just anterior to the ventral sucker. The first positive observations of the post-pharyngeal region of this system obtained by the writer were in the study of a living ten-day-old metacercaria, in which it was possible to see a single tube extending posteriorly. Liquid, with suspended granules, was being forced back and forth in this tube. In the study of metacercariæ 30 days old and older, it was easy to see the exact nature of the entire system (see Fig. 11) in both living and mounted specimens. The esophagus extends posteriorly, dorsal to the gonads, and in the region posterior to the cirrus pouch it gives rise to two large bulb-like intestinal ceca.

The reproductive organs, as they appear in metacercariæ 35 to 49 days old, are shown in Fig. 11. The testes are located exactly as Linton described them in the cercariæum. The vasa deferentia are short, slightly coiled tubules leading to the cirrus pouch, in which they unite to form a seminal vesicle. From the vesicle an ejaculatory duct traverses the cirrus sac and the genital pore is situated on the left margin of the body at the level of the aperture of the acetabulum. The ovary is situated just anterior to the excretory vesicle, as reported by Miller and Northup. The oviduct is short and communicates with a seminal receptacle on the right side of the body, while the uterus extends as a long tortuous canal to the metraterm which is parallel to the cirrus sac and opens at the genital pore. The vitellaria were not located with certainty.

Measurements of metacercariæ of various ages (dissected from their cysts) are given in Table III. These measurements were used in part in constructing Figs. 10 and 11. Comparison with Table I shows the extent of development.

Adult Host

Only one attempt was made to determine the host of the adult trematode and this experiment was unsuccessful. The host of the

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EXPLANATION OF PLATE II

FIG. 7. A diagrammatic sketch of a ten-day-old metacercaria emerging from its cyst which has been crushed by slight pressure.

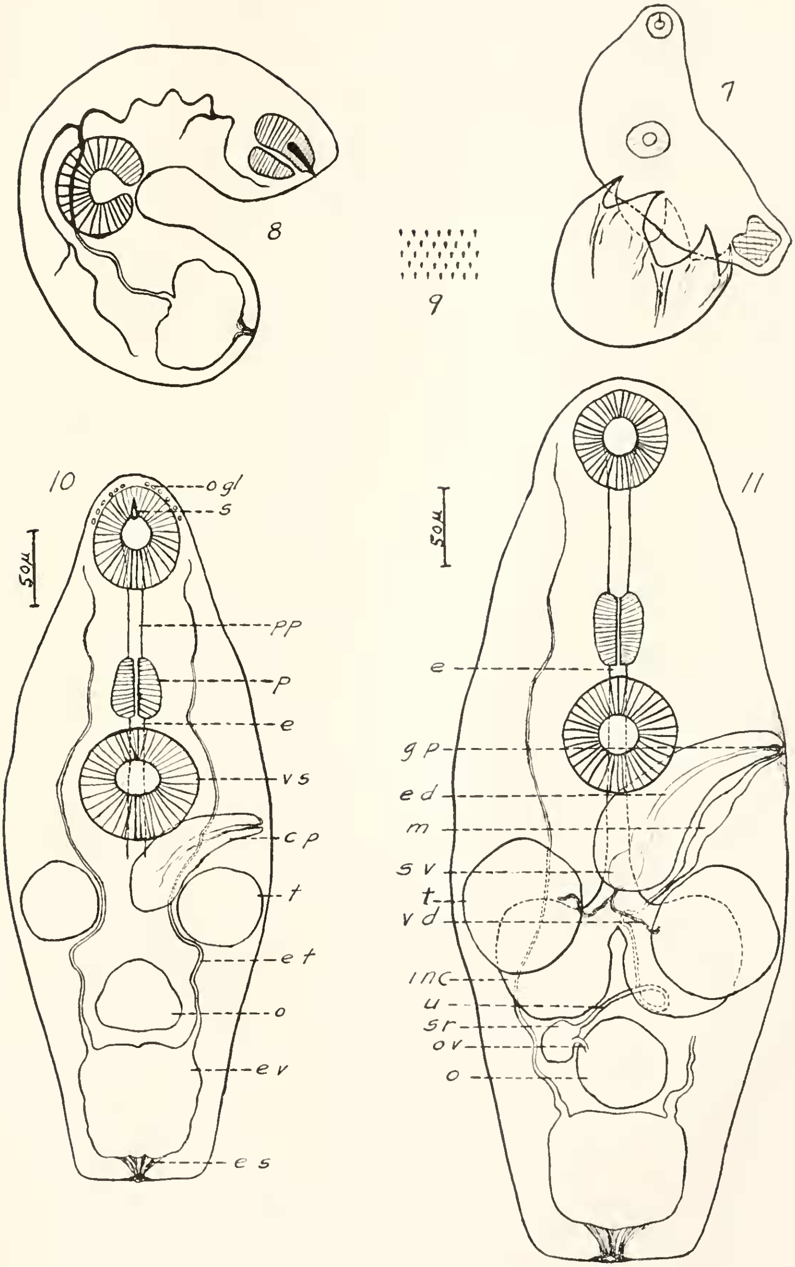
FIG. 8. Lateral view of a two-day-old metacercaria of *Cercariæum lintoni,* showing the relationship of the most prominent structures of that stage.

FIG. 9. Diagram to show the plan of arrangement of the spines which cover the surface of the body of *C. lintoni* and its metacercaria.

FIG. 10. Diagrammatic drawing of 18-day-old metacercaria of *C. lintoni.* Ventral view.

FIG. 11. Diagrammatic drawing of 45-day-old metacercaria of *C. lintoni.* Ventral view.

PLATE II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adult is, presumably, a fish which eats *Nereis*. Since the eel and *Nereis* have been observed in the same habitat in the Woods Hole region, since eels are believed to eat clam worms, and since the eel is a hardy animal which will withstand laboratory conditions, this species was selected for the experiment. On August 7, 1931 two infested *Nereis virens* (one containing 45- and the other 38-day-old metacercariæ) were fed to an eel, *Anguilla chrisypa* Raf. The eel died three weeks later but no trematodes were found.

Discussion

When it was discovered that *Cercariæum lintoni* would develop in *Nereis virens*, it appeared probable that advanced metacercarial stages

Table III

Measurements of metacercariæ of Cercariæum lintoni M. and N. All measurements are in millimeters; all measurements made from living specimens.

	13 days old	18 days old	27 days old	45 days old
Length	.42	.46	.47	.575
Width	.15	.1725	.24	.215
Oral sucker, length	.075	.07	.07	.065
" " width	.06	.06	.0625	.06
Ventral sucker, length	.07	.075	.075	.075
" " width	.085	.08	.075	.075
Pharynx, length	.035	.04	.042	.0484
" width	.032	.035	.038	.0374
Testis, length	.065	.06	.065	.08
" width	.06	.06	.07	.085
Ovary, length	.05	.05	.055	.06
" width	.06	.06	.06	.06
Excretory vesicle, length	.075	.075	.07	.075
" " width	.07	.08	.09	.09
Stylet, length	.0156	.0143	.0066	—
" width	.0022	.0022	.002	—

would be sufficiently mature so that comparison of them with descriptions of marine trematodes might suggest the adult form. It was thought that at least the family to which they belong could be determined. This hope has not, however, been realized. Examination of the papers by Looss (1894), Lühe (1909), Odhner (1911), Linton (1898, 1899, 1904, 1907, 1910), Manter (1926, 1931), and Fuhrmann (1928) has failed to disclose a trematode that could be considered as the adult form of *C. lintoni.*

Palombi (1930) worked out the life history of a tailless cercaria at Naples and found that it developed into *Diphterostomum brusinæ* (Stossich), a member of the family Zoogonidæ. He compared the cercaria of

D. brusinæ with *Cercariæum lintoni* and concluded that they were closely related species. He stated: "*Cercariæum lintoni* Miller e Northup parassita die *Nassa obsoleta* (Say), presenta sensibili affinitá con la cercariæ di *Diphterostomum brusinæ* Stoss. parassita di *Nassa mutabilis* L., *N. reticulata* L. e *Natica Poliana* Delle Chiaie. Non e improbabile che essa rappresenti la forma larvale die una specie della famiglia *Zoogonidæ* Odhner." Table IV gives a detailed comparison of the two

TABLE IV

Comparison of the cercariæum of Diphterostomum brusinæ Stossich and Cercariæum lintoni Miller and Northup

	Cercariæum of *D. brusinae* Stoss.	*Cercariæum lintoni* M. and N.
Length	.23 mm.	.32 mm.
Width	.10 mm.	.102 mm.
Size of ventral sucker	Considerably larger than oral sucker.	Slightly larger than oral sucker.
Position of ventral sucker	Just posterior to middle region.	In middle region.
Lateral view of v. sucker	Shows two peculiar "prominences."	Without peculiar prominences (Fig. 8).
Stylet	Present.	Present.
Pre-pharynx	None.	Long.
Pharynx	Just posterior to oral sucker.	Just anterior to ventral sucker.
Esophagus	Long.	Rather long.
Intestinal ceca	Slender and short.	Short, bulb-like.
Testes	Just posterior to ventral sucker.	Just posterior to ventral sucker.
Genital pore	Median, slightly anterior.	Lateral, left, just posterior to ventral sucker.
Sporocyst	In *Nassa mutabilis*.	In *Nassa obsoleta*.
Free living stage	None.	Emerges from snail; active for hours.
Encystment	In snail host; in or out of sporocyst.	In parapodia of *Nereis virens*.
Mode of entrance into next host after snail	Eaten by fish.	Penetrate parapodia of *Nereis virens*.

species and indicates that they are not as closely related as Palombi believed.

Palombi suggested that *Cercariæum lintoni* may be the cercarial form of a species of *Deretrema* Linton, Family *Zoogonidæ*. The morphological characters of the metacercaria show, however, that the species does not belong to the genus *Deretrema*. In the arrangement of reproductive organs it agrees with members of the family *Zoogonidæ*, but the digestive system is very different. The digestive system of the metacercaria resembles that of *Saccocoelium* Looss (Family *Haploporidæ*), but the reproductive organs do not correspond to those of the *Haploporidæ*. It is, accordingly, impossible to assign *C. lintoni* to any family as they are at present characterized, and determination of the systematic position of the species must await further knowledge.

Summary

The description of *Cercariæum lintoni* Miller and Northup as given by earlier workers is supplemented and emended in the light of knowledge obtained by comparing the cercariæum with its metacercarial stage, developed in experimental animals. The true nature of the digestive and reproductive systems is made clear.

Cercariæum lintoni penetrated the parapodia of *Nereis virens*, encysted, and developed into the metacercarial stage. Although the cercaria would penetrate and encyst in other invertebrates, the clam worm was the only form in which development was secured. *Nereis virens* appears to be a natural host. The metacercaria develops adult features as the larval characters disappear.

It is difficult to determine the relationship of this species to other trematode groups; the digestive system is similar to that of the species of the family *Haploporidæ*, but the arrangement of the reproductive organs is very different and resembles that of the *Zoogonidæ*. Palombi's suggestions concerning the relations of the species are discussed.

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